METHODS AND COMPOSITIONS FOR TREATING RENAL FAILURE

FIELD OF THE INVENTION

The invention relates to compositions that can improve renal function and are useful as dietary supplements (e.g., health drinks) or medication. These compositions contain yeast cells obtainable by growth in electromagnetic fields with specific frequencies and field strengths.

BACKGROUND OF THE INVENTION

Renal failure is a disease state in which renal functions are damaged severely such that internal environment of the living body can no longer be maintained in normal conditions. In particular, acute renal failure involves a sudden loss of the kidneys' ability to excrete wastes, concentrate urine, and conserve electrolytes. Causes of acute renal failure include acute tubular necrosis (ATN), myoglobinuria (myoglobin in the urine), infections such as acute pyelonephritis or septicemia, urinary tract obstruction such as a narrowing of the urinary tract (stricture), tumor, kidney stones, nephrocalcinosis, enlarged prostate with subsequent acute bilateral obstructive uropath, severe acute nephritic syndrome, disorders of the blood, malignant hypertension, and autoimmune disorders such as scleroderma. Other causes such as poisons and trauma, for example a direct and forceful blow to the kidneys, can also lead to renal failure.

Chronic renal failure is a gradual loss of kidney functions and usually occurs over a number of years as the internal structures of the kidney are slowly destroyed. Causative diseases include glomerulonephritis of any type, polycystic kidney disease, diabetes mellitus, hypertension, Alport syndrome, reflux nephropathy, obstructive uropathy, kidney stones and infection, and analgesic nephropathy. Chronic renal failure results in the accumulation of fluid and waste products in the body, causing azotemia and uremia.

Therapeutic agents for acute renal failure include loop diuretics and osmotic diuretics, which are used in expectation of recovery of renal functions by increasing the flow in kidney tubules so as to wash away casts formed in the tubules and thereby prevent obstruction of the tubules. Agents for chronic renal failure include imidazole angiotensin-II (AII) receptor antagonists and anipamil. However, depending on the manner of use, these agents present the risk of inviting hearing disorders and the even more severe adverse side effects of heart failure and pulmonary edema.

SUMMARY OF THE INVENTION

This invention is based on the discovery that certain yeast cells can be activated by electromagnetic fields having specific frequencies and field strengths to produce substances useful in ameliorating renal failure. Compositions comprising these activated yeast cells can be used as medication or as dietary supplements, in the form of health drinks or dietary pills (tablets or powder). For instance, these compositions can be used to treat renal failure in a subject (e.g., a human subject) as indicated by their improved diuretic effect and/or lowered blood urea nitrogen, proteinuria, and/or serum creatinine levels.

This invention embraces a composition comprising a plurality of yeast cells that have been cultured in an alternating electric field having a frequency in the range of about 9500 to 13000 MHz (e.g., 9750-10500, 12000-12500 and/or 12600-12980 MHz) and a field strength in the range of about 220 to 480 mV/cm (e.g., 250-270, 260-280, 280-305, 290-310, 315-335, 325-345, 350-370, 370-390, 380-400, 380-420, and/or 430-450 mV/cm). The yeast cells are cultured for a period of time sufficient to activate said plurality of yeast cells to

treat kidney diseases in a subject. In one embodiment, the frequency and/or the field strength of the alternating electric field can be altered within the aforementioned ranges during said period of time. In other words, the yeast cells are exposed to a series of electromagnetic fields. An exemplary period of time is about 130-230 hours (e.g., 169-193 hours).

Also included in this invention is a composition comprising a plurality of yeast cells that have been cultured under acidic conditions in an alternating electric field having a frequency in the range of about 12000 to 13000 MHz (e.g., 12500-13000 MHz) and a field strength in the range of about 300 to 420 mV/cm (e.g., 350-370 and/or 370-390 mV/cm). In one embodiment, the yeast cells are exposed to a series of electromagnetic fields. An exemplary period of time is about 50-100 hours (e.g., 57-73 hours).

Yeast cells that can be included in this composition can be derived from parent strains publically available from the China General Microbiological Culture Collection Center ("CGMCC"), China Committee for Culture Collection of Microorganisms, Institute of Microbiology, Chinese Academy of Sciences, Haidian, P.O. Box 2714, Beijing, 100080, China. Useful yeast species include, but are not limited to, those commonly used in food and pharmaceutical industries, such as *Saccharomyces cerevisiae* (e.g., Hansen and Hansen Var. ellipsoideus), *Saccharomyces carlsbergensis, Saccharomyces rouxii*, *Saccharomyces sake*, *Saccharomyces uvarum*, *Saccharomyces sp.*, *Schizosaccharomyces pombe*, *Rhodotorula aurantiaca* and *Rhodotorula rubar*. For instance, the yeast cells can be of the strain *Saccharomyces cerevisiae* Hansen AS2.16, AS2.112 or AS2.504, *Saccharomyces sp.* AS2.311, *Schizosaccharomyces pombe* Lindner AS2.274, *Saccharomyces sake* Yabe ACCC2045, *Saccharomyces uvarum* Beijer IFFI1207, *Saccharomyces rouxii* Boutroux AS2.370, *Saccharomyces cerevisiae* Hansen Var. ellipsoideus AS2.612, *Saccharomyces carlsbergensis* Hansen AS2.417, or *Rhodotorula rubar* (Demme) Lodder AS2.105. Other useful yeast strains are illustrated in Table 1.

This invention further embraces a composition comprising a plurality of yeast cells, wherein said plurality of yeast cells have been activated to

treat kidney diseases in a subject. Included in this invention are also methods of making the above compositions.

As used herein, a subject includes a human and veterinary subject.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Exemplary methods and materials are described below, although methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention. All publications and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. The materials, methods, and examples are illustrative only and not intended to be limiting. Throughout this specification and claims, the word "comprise," or variations such as "comprises" or "comprising" will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a schematic diagram showing an exemplary apparatus for activating yeast cells using electromagnetic fields. 1: yeast culture; 2: container; 3: power supply.

Fig. 2 is a schematic diagram showing an exemplary apparatus for making yeast compositions of the invention. The apparatus comprises a signal generator (such as models 83721B and 83741A manufactured by HP) and interconnected containers A, B and C.

DETAILED DESCRIPTION OF THE INVENTION

This invention is based on the discovery that certain yeast strains can be activated by electromagnetic fields ("EMF") having specific frequencies and field strengths to become highly efficient in producing substances that restore urine secretion and/or lower blood urea nitrogen, proteinuria and/or serum

creatinine levels in a subject. Compositions containing these activated yeast cells are thus useful in treating kidney diseases. Yeast compositions containing activated yeast cells can be used as medication or as dietary supplements, in the form of health drinks or dietary pills (tablets or powder).

Since the activated yeast cells contained in the yeast compositions have been cultured to endure acidic conditions (pH 2.5-4.2), these cells can survive the gastric environment and pass on to the intestines. Once in the intestines, the yeast cells are ruptured by various digestive enzymes, and the active substances in treatment of kidney diseases are released and readily absorbed.

I. **Yeast Strains Useful in the Invention**

The types of yeasts useful in this invention include, but are not limited to, yeasts of the genera *Saccharomyces*, *Schizosaccharomyces*, and *Rhodotorula*.

Exemplary species within the above-listed genera include, but are not limited to, those illustrated in Table 1. Yeast strains useful for this invention can be obtained from laboratory cultures, or from publically accessible culture depositories, such as CGMCC and the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209. Non-limiting examples of useful strains (with accession numbers of CGMCC) are *Saccharomyces cerevisiae* Hansen AS2.16, AS2.112 and AS2.504, *Saccharomyces sp.* AS2.311, *Schizosaccharomyces pombe* Lindner AS2.274, *Saccharomyces sake* Yabe ACCC2045, *Saccharomyces uvarum* Beijer IFFI1207, *Saccharomyces rouxii* Boutroux AS2.370, *Saccharomyces cerevisiae* Hansen Var. ellipsoideus AS2.612, *Saccharomyces carlsbergensis* Hansen AS2.417, and *Rhodotorula rubar* (Demme) Lodder AS2.105. Other useful yeast strains are illustrated in Table 1.

The preparation of the yeast compositions of this invention is not limited to starting with a pure strain of yeast. A yeast composition of the invention may be produced by culturing a mixture of yeast cells of different species or strains. The ability of any activated species or strain of yeasts to treat kidney diseases can be readily tested by methods known in the art. See, for instance, Examples 1 and 2.

Table 1 Exemplary Yeast Strains

Saccharomyces cerevisiae Hansen				
ACCC2034	ACCC2035	ACCC2036	ACCC2037	ACCC2038
ACCC2039	ACCC2040	ACCC2041	ACCC2042	AS2. 1
AS2. 4	AS2. 11	AS2. 14	AS2. 16	AS2. 56
AS2. 69	AS2. 70	AS2. 93	AS2. 98	AS2. 101
AS2. 109	AS2. 110	AS2. 112	AS2. 139	AS2. 173
AS2. 174	AS2. 182	AS2. 196	AS2. 242	AS2. 336
AS2. 346	AS2. 369	AS2. 374	AS2. 375	AS2. 379
AS2. 380	AS2. 382	AS2. 390	AS2. 393	AS2. 395
AS2. 396	AS2. 397	AS2. 398	AS2. 399	AS2. 400
AS2. 406	AS2. 408	AS2. 409	AS2. 413	AS2. 414
AS2. 415	AS2. 416	AS2. 422	AS2. 423	AS2. 430
AS2. 431	AS2. 432	AS2. 451	AS2. 452	AS2. 453
AS2. 458	AS2. 460	AS2. 463	AS2. 467	AS2. 486
AS2. 501	AS2. 502	AS2. 503	AS2. 504	AS2. 516
AS2. 535	AS2. 536	AS2. 558	AS2. 560	AS2. 561
AS2. 562	AS2. 576	AS2. 593	AS2. 594	AS2. 614
AS2. 620	AS2. 628	AS2. 631	AS2. 666	AS2. 982
AS2. 1190	AS2. 1364	AS2. 1396	IFFI1001	IFFI1002
IFFI1005	IFFI1006	IFFI1008	IFFI1009	IFFI1010
IFFI1012	IFFI1021	IFFI1027	IFFI1037	IFFI1042
IFFI1043	IFFI1045	IFFI1048	IFFI1049	IFFI1050
IFFI1052	IFFI1059	IFFI1060	IFFI1062	IFFI1063
IFFI1202	IFFI1203	IFFI1206	IFFI1209	IFFI1210
IFFI1211	IFFI1212	IFFI1213	IFFI1214	IFFI1215
IFFI1220	IFFI1221	IFFI1224	IFFI1247	IFFI1248
IFFI1251	IFFI1270	IFFI1277	IFFI1287	IFFI1289
IFFI1290	IFFI1291	IFFI1292	IFFI1293	IFFI1297
IFFI1300	IFFI1301	IFFI1302	IFFI1307	IFFI1308
IFFI1309	IFFI1310	IFFI1311	IFFI1331	IFFI1335

IFFI1336	IFFI1337	IFFI1338	IFFI1339	IFFI1340
IFFI1345	IFFI1348	IFFI1396	IFFI1397	IFFI1399
IFFI1411	IFFI1413	IFFI1441	IFFI1443	
Saccharomyces cerevisiae Hansen Var. ellipsoideus (Hansen) Dekker				
ACCC2043	AS2.2	AS2.3	AS2.8	AS2.53
AS2.163	AS2.168	AS2.483	AS2.541	AS2.559
AS2.606	AS2.607	AS2.611	AS2.612	
Saccharomyces chevalieri Guilliermond				
AS2.131	AS2.213			
Saccharomyces delbrueckii				
AS2.285				
Saccharomyces delbrueckii Lindner ver. mongolicus (Saito) Lodder et van Rij				
AS2.209	AS2.1157			
Saccharomyces exiguous Hansen				
AS2.349	AS2.1158			
Saccharomyces fermentati (Saito) Lodder et van Rij				
AS2.286	AS2.343			
Saccharomyces logos van laer et Denamur ex Jorgensen				
AS2.156	AS2.327	AS2.335		
Saccharomyces mellis (Fabian et Quinet) Lodder et kreger van Rij				
AS2.195				
Saccharomyces mellis Microellipsoides Osterwalder				
AS2.699				
Saccharomyces oviformis Osteralder				
AS2.100				
Saccharomyces rosei (Guilliermond) Lodder et Kreger van Rij				
AS2.287				
Saccharomyces rouxii Boutroux				
AS2.178	AS2.180	AS2.370	AS2.371	
Saccharomyces sake Yabe				

ACCC2045				
Candida arborea				
AS2.566				
Candida lambica (Lindner et Genoud) van. Uden et Buckley				
AS2.1182				
Candida krusei (Castellani) Berkhout				
AS2.1045				
Candida lipolytica (Harrison) Diddens et Lodder				
AS2.1207	AS2.1216	AS2.1220	AS2.1379	AS2.1398
AS2.1399	AS2.1400			
Candida parapsilosis (Ashford) Langeron et Talice Var. intermedia Van Rij et Verona				
AS2.491				
Candida parapsilosis (Ashford) Langeron et Talice				
AS2.590				
Candida pulcherrima (Lindner) Windisch				
AS2.492				
Candida rugousa (Anderson) Diddens et Lodder				
AS2.511	AS2.1367	AS2.1369	AS2.1372	AS2.1373
AS2.1377	AS2.1378	AS2.1384		
Candida tropicalis (Castellani) Berkhout				
ACCC2004	ACCC2005	ACCC2006	AS2.164	AS2.402
AS2.564	AS2.565	AS2.567	AS2.568	AS2.617
AS2.637	AS2.1387	AS2.1397		
Candida utilis Henneberg Lodder et Kreger Van Rij				
AS2.120	AS2.281	AS2.1180		
Crebrothecium ashbyii (Guillermond) Routein (*Eremothecium ashbyii* Guilliermond)				
AS2.481	AS2.482	AS2.1197		
Geotrichum candidum Link				

ACCC2016	AS2.361	AS2.498	AS2.616	AS2.1035
AS2.1062	AS2.1080	AS2.1132	AS2.1175	AS2.1183
Hansenula anomala (Hansen)H et P sydow				
ACCC2018	AS2.294	AS2.295	AS2.296	AS2.297
AS2.298	AS2.299	AS2.300	AS2.302	AS2.338
AS2.339	AS2.340	AS2.341	AS2.470	AS2.592
AS2.641	AS2.642	AS2.782	AS2.635	AS2.794
Hansenula arabitolgens Fang				
AS2.887				
Hansenula jadinii (A. et R Sartory Weill et Meyer) Wickerham				
ACCC2019				
Hansenula saturnus (Klocker) H et P sydow				
ACCC2020				
Hansenula schneggii (Weber) Dekker				
AS2.304				
Hansenula subpelliculosa Bedford				
AS2.740	AS2.760	AS2.761	AS2.770	AS2.783
AS2.790	AS2.798	AS2.866		
Kloeckera apiculata (Reess emend. Klocker) Janke				
ACCC2022	ACCC2023	AS2.197	AS2.496	AS2.714
ACCC2021	AS2.711			
Lipomycess starkeyi Lodder et van Rij				
AS2.1390	ACCC2024			
Pichia farinosa (Lindner) Hansen				
ACCC2025	ACCC2026	AS2.86	AS2.87	AS2.705
AS2.803				
Pichia membranaefaciens Hansen				
ACCC2027	AS2.89	AS2.661	AS2.1039	
Rhodosporidium toruloides Banno				
ACCC2028				

Rhodotorula glutinis (Fresenius) Harrison				
AS2.2029	AS2.280	ACCC2030	AS2.102	AS2.107
AS2.278	AS2.499	AS2.694	AS2.703	AS2.704
AS2.1146				
Rhodotorula minuta (Saito) Harrison				
AS2.277				
Rhodotorula rubar (Demme) Lodder				
AS2.21	AS2.22	AS2.103	AS2.105	AS2.108
AS2.140	AS2.166	AS2.167	AS2.272	AS2.279
AS2.282	ACCC2031			
Rhodotorula aurantiaca (Saito) Lodder				
AS2.102	AS2.107	AS2.278	AS2.499	AS2.694
AS2.703	AS2.1146			
Saccharomyces carlsbergensis Hansen				
AS2.113	ACCC2032	ACCC2033	AS2.312	AS2.116
AS2.118	AS2.121	AS2.132	AS2.162	AS2.189
AS2.200	AS2.216	AS2.265	AS2.377	AS2.417
AS2.420	AS2.440	AS2.441	AS2.443	AS2.444
AS2.459	AS2.595	AS2.605	AS2.638	AS2.742
AS2.745	AS2.748	AS2.1042		
Saccharomyces uvarum Beijer				
IFFI1023	IFFI1032	IFFI1036	IFFI1044	IFFI1072
IFFI1205	IFFI1207			
Saccharomyces willianus Saccardo				
AS2.5 AS2.7	AS2.119	AS2.152	AS2.293	
AS2.381	AS2.392	AS2.434	AS2.614	AS2.1189
Saccharomyces sp.				
AS2.311				
Saccharomycodes ludwigii Hansen				
ACCC2044	AS2.243	AS2.508		

Saccharomycodes sinenses Yue				
AS2.1395				
Schizosaccharomyces octosporus Beijerinck				
ACCC2046	AS2.1148			
Schizosaccharomyces pombe Lindner				
ACCC2047	ACCC2048	AS2.214	AS2.248	AS2.249
AS2.255	AS2.257	AS2.259	AS2.260	AS2.274
AS2.994	AS2.1043	AS2.1149	AS2.1178	IFFI1056
Sporobolomyces roseus Kluyver et van Niel				
ACCC2049	ACCC2050	AS2.19	AS2.962	AS2.1036
ACCC2051	AS2.261	AS2.262		
Torulopsis candida (Saito) Lodder				
AS2.270	ACCC2052			
Torulopsis famta (Harrison) Lodder et van Rij				
ACCC2053	AS2.685			
Torulopsis globosa (Olson et Hammer) Lodder et van Rij				
ACCC2054	AS2.202			
Torulopsis inconspicua Lodder et Kreger van Rij				
AS2.75				
Trichosporon behrendii Lodder et Kreger van Rij				
ACCC2056	AS2.1193			
Trichosporon capitatum Diddens et Lodder				
ACCC2056	AS2.1385			
Trichosporon cutaneum (de Beurm et al.) Ota				
ACCC2057	AS2.25	AS2.570	AS2.571	AS2.1374
Wickerhamia fluorescens (Soneda) Soneda				
ACCC2058	AS2.1388			

II. <u>Application of Electromagnetic Fields</u>

An electromagnetic field useful in this invention can be generated and applied by various means well known in the art. For instance, the EMF can be generated by applying an alternating electric field or an oscillating magnetic field.

Alternating electric fields can be applied to cell cultures through electrodes in direct contact with the culture medium, or through electromagnetic induction. See, e.g., Fig. 1. Relatively high electric fields in the medium can be generated using a method in which the electrodes are in contact with the medium. Care must be taken to prevent electrolysis at the electrodes from introducing undesired ions into the culture and to prevent contact resistance, bubbles, or other features of electrolysis from dropping the field level below that intended. Electrodes should be matched to their environment, for example, using Ag-AgCl electrodes in solutions rich in chloride ions, and run at as low a voltage as possible. For general review, see Goodman et al., *Effects of EMF on Molecules and Cells*, International Review of Cytology, A Survey of Cell Biology, Vol. 158, Academic Press, 1995.

The EMFs useful in this invention can also be generated by applying an oscillating magnetic field. An oscillating magnetic field can be generated by oscillating electric currents going through Helmholtz coils. Such a magnetic field in turn induces an electric field.

The frequencies of EMFs useful in this invention range from about 9500-13000 MHz (e.g., 9750-10500, 12000-12500 and/or 12600-12980 MHz). Exemplary frequencies are 10102, 10114, 12237, 12877, and 12895 MHz. The field strength of the electric field useful in this invention ranges from about 220 to 480 mV/cm (e.g., 250-270, 260-280, 280-305, 290-310, 315-335, 325-345, 350-370, 370-390, 380-400, 380-420, and/or 430-450 mV/cm). Exemplary field strengths are 250, 278, 280, 307, 321, 334, 352, 353, 372, 377, 385, 406, and 438 mV/cm.

When a series of EMFs are applied to a yeast culture, the yeast culture can remain in the same container while the same set of EMF generator and emitters is used to change the frequency and/or field strength. The EMFs in the series can each have a different frequency or a different field strength; or a different frequency and a different field strength. Such frequencies and field

strengths are preferably within the above-described ranges. Although any practical number of EMFs can be used in a series, it may be preferred that the yeast culture be exposed to a total of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or more EMFs in a series. In one embodiment, the yeast culture is exposed to a series of EMFs, wherein the frequency of the electric field is alternated in the range of about 9750-10500, 12000-12500 and 12600-12980 MHz.

Although the yeast cells can be activated after even a few hours of culturing in the presence of an EMF, it may be preferred that the activated yeast cells be allowed to multiply and grow in the presence of the EMF(s) for a total of 30-200 hours (e.g., 35-100 hours).

Fig. 1 illustrates an exemplary apparatus for generating alternating electric fields. An electric field of a desired frequency and intensity is generated by an AC source (3) capable of generating an alternating electric field, preferably in a sinusoidal wave form, in the frequency range of 10 to 20,000 MHz. Signal generators capable of generating signals with a narrower frequency range can also be used. If desirable, a signal amplifier can also be used to increase the output. The activation container (2) can be made from non-conductive material, for example, plastics, glass steel, ceramic, and combinations thereof. The wire connecting the activation container (2) and the signal generator (3) is preferably a high frequency coaxial cable with a transmission frequency of at least 30 GHz.

The alternating electric field can be applied to the culture by a variety of means, including placing the yeast culture (1) in close proximity to the signal emitters such as a metal wire or tube capable of transmitting EMFs. The metal wire or tube can be made of red copper, and be placed inside the container (2), reaching as deep as 3-30 cm. For example, if the fluid in the container (2) has a depth of 15-20 cm, 20-30 cm, 30-50 cm, 50-70 cm, 70-100 cm, 100-150 cm or 150-200 cm, the metal wire can be 3-5 cm, 5-7 cm, 7-10 cm, 10-15 cm, 15-20 cm, 20-30 cm and 25-30 cm from the bottom of the container (2), respectively. The number of electrode wires used depends on the volume of the culture as well as the diameter of the wires. The number of metal wires/tubes used can be from 1 to 10 (e.g., 2 to 3). It is recommended, though not mandated, that for a culture having a volume up to 10 L, metal wires/tubes having a diameter of 0.5 to 2.0 mm be used.

For a culture having a volume between 10 L and 100 L, metal wires/tubes having a diameter of 3.0 to 5.0 mm can be used. For a culture having a volume in the range of 100-1000 L, metal wires/tubes having a diameter of 6.0 to 15.0 mm can be used. For a culture having a volume greater than 1000 L, metal wires/tubes having a diameter of 20.0 to 25.0 mm can be used.

In one embodiment, the electric field is applied by electrodes submerged in the culture (1). In this embodiment, one of the electrodes can be a metal plate placed on the bottom of the container (2), and the other electrode can comprise a plurality of electrode wires evenly distributed in the culture (1) so as to achieve even distribution of the electric field energy. The number of electrode wires used depends on the volume of the culture as well as the diameter of the wires.

III. Culture Media

Culture media useful in this invention contain sources of nutrients assimilable by yeast cells. Complex carbon-containing substances in a suitable form, such as carbohydrates (e.g., sucrose, glucose, fructose, dextrose, maltose, xylose, cellulose, starches, etc.) and coal, can be the carbon sources for yeast cells. The exact quantity of the carbon sources utilized in the medium can be adjusted in accordance with the other ingredients of the medium. In general, the amount of carbohydrates varies between about 0.1% and 10% by weight of the medium and preferably between about 0.1% and 5% (e.g., about 2%). These carbon sources can be used individually or in combination. Amino acid-containing substances in suitable form (e.g., beef extract and peptone) can also be added individually or in combination. In general, the amount of amino acid containing substances varies between about 0.1% and 0.5% by weight of the medium and preferably between about 0.1% and 0.3% (e.g., about 0.25%). Among the inorganic salts which can be added to the culture medium are the customary salts capable of yielding sodium, potassium, calcium, phosphate, sulfate, carbonate, and like ions. Non-limiting examples of nutrient inorganic salts are $(NH_4)_2HPO_4$, KH_2PO_4, K_2HPO_4, $CaCO_3$, $MgSO_4$, NaCl, and $CaSO_4$.

IV. Electromagnetic Activation of Yeast Cells

To activate or enhance the ability of yeast cells to produce substances beneficial for renal functions (e.g., increasing urine secretion and/or lowering of blood urea nitrogen, proteinuria and/or serum creatinine levels), yeast cells of this invention can be activated by being cultured in an appropriate medium under sterile conditions at 20°C-38°C, preferably at 28-32°C (e.g., 30°C) for a sufficient amount of time, e.g., 130-230 hours (e.g., 169-193 hours), in an alternating electric field or a series of alternating electric fields as described above.

An exemplary culture medium is made by mixing 1000 ml of distilled water with 18 g of mannitol, 40 μg of vitamin B_3, 30 μg of vitamin B_{12}, 10 μg of vitamin H, 35 ml of fetal bovine serum, 0.20 g of KH_2PO_4, 0.25 g of $MgSO_4{\cdot}7H_2O$, 0.3 g of NaCl, 0.2 g of $CaSO_4{\cdot}2H_2O$, 4.0 g of $CaCO_3{\cdot}5H_2O$, and 2.5 g of peptone.

An exemplary set-up of the culturing process is depicted in Fig. 1. Untreated yeast cells are added to a culture medium at 1×10^8 cells per 1000 ml of the culture medium. The yeast cells may be *Saccharomyces cerevisiae* Hansen AS2.504 or AS2.16, or may be selected from any of the strains listed in Table 1. An exemplary activation process of the yeast cells involves the following sequence: the yeast cells are grown in the culture medium for 26-30 hours (e.g., 28 hours) at 28-32°C and then exposed to (1) an alternating electric field having a frequency of 10102 MHz and a field strength in the range of 260-280 mV/cm (e.g., 278 mV/cm) for 14-18 hours (e.g., 16 hours); (2) then to an alternating electric field having a frequency of 10114 MHz and a field strength in the range of 290-310 mV/cm (e.g., 307 mV/cm) for 34-38 hours (e.g., 36 hours); (3) then to an alternating electric field having a frequency of 12237 MHz and a field strength in the range of 325-345 mV/cm (e.g., 334 mV/cm) for 42-46 hours (e.g., 44 hours); (4) then to an alternating electric field having a frequency of 12877 MHz and a field strength in the range of 350-370 mV/cm (e.g., 353 mV/cm) for 37-41 hours (e.g., 39 hours); and (5) finally to an alternating electric field having a frequency of 12895 MHz and a field strength in the range of 280-305 mV/cm (e.g., 280 mV/cm) for 16-20 hours (e.g., 18 hours). The activated yeast cells are then recovered from the culture medium by various methods known in the art, dried (e.g., by

lyophilization) and stored at about 4°C in powder form. The resultant yeast powder preferably contains more than 10^{10} cells/g.

Subsequently, the activated yeast cells can be measured for their ability to treat kidney diseases (e.g., improve kidney functions) using standard methods known in the art, such as those described in Section VII.

V. Acclimatization of Yeast Cells To the Gastric Environment

Because the activated yeast cells of this invention must pass through the stomach before reaching the small intestine, where the effective components are released from these yeast cells, it is preferred that these yeasts be cultured under acidic conditions so as to acclimatize the cells to the gastric juice. This acclimatization process results in better viability of the yeast cells in the acidic gastric environment.

To achieve this, the yeast powder containing activated yeast cells can be mixed with a highly acidic acclimatizing culture medium at 10 g (containing more than 10^{10} activated cells per gram) per 1000 ml. The yeast mixture can then be cultured first in the presence of an alternating electric field having a frequency of 12877 MHz and a field strength in the range of 370-390 mV/cm (e.g., 377 mV/cm) at about 28 to 32°C for 42-48 hours (e.g., 46 hours). The resultant yeast cells can then be further incubated in the presence of an alternating electric field having a frequency of 12895 MHz and a field strength in the range of 350-370 mV/cm (e.g., 352 mV/cm) at about 28 to 32°C for 15-25 hours (e.g., 20 hours). The resulting acclimatized yeast cells are then recovered from the culture medium by various methods known in the art and are dried and stored either in powder form ($\geq 10^{10}$ cells/g) at room temperature or in vacuum at 0-4°C.

An exemplary acclimatizing culture medium is made by mixing 700 ml fresh pig gastric juice and 300 ml wild Chinese hawthorn extract. The pH of acclimatizing culture medium is adjusted to 2.5 with 0.1 M hydrochloric acid (HCl) and 0.2 M potassium hydrogen phthalate ($C_6H_4(COOK)COOH$). The fresh pig gastric juice is prepared as follows. At about 4 months of age, newborn Holland white pigs are sacrificed, and the entire contents of their stomachs are retrieved and mixed with 2000 ml of water under sterile conditions. The mixture is

then allowed to stand for 6 hours at 4°C under sterile conditions to precipitate food debris. The supernatant is collected for use in the acclimatizing culture medium. To prepare the wild Chinese hawthorn extract, 500 g of fresh wild Chinese hawthorn is dried under sterile conditions to reduce water content (≤8%). The dried fruit is then ground (≥20 mesh) and added to 1500 ml of sterile water. The hawthorn slurry is allowed to stand for 6 hours at 4°C under sterile conditions. The hawthorn supernatant is collected to be used in the acclimatizing culture medium.

VI. <u>Manufacture of Yeast Compositions</u>

To prepare the yeast compositions of the invention, an apparatus depicted in Fig. 2 or an equivalent thereof can be used. This apparatus includes three containers, a first container (A), a second container (B), and a third container (C), each equipped with a pair of electrodes (4). One of the electrodes is a metal plate placed on the bottom of the containers, and the other electrode comprises a plurality of electrode wires evenly distributed in the space within the container to achieve even distribution of the electric field energy. All three pairs of electrodes are connected to a common signal generator.

The culture medium used for this purpose is a mixed fruit extract solution containing the following ingredients per 1000 L: 300 L of wild Chinese hawthorn extract, 300 L of jujube extract, 300 L of *Schisandra chinensis (Turez) Baill* seeds extract, and 100 L of soy bean extract. To prepare hawthorn, jujube and *Schisandra chinensis (Turez) Baill* seeds extracts, the fresh fruits are washed and dried under sterile conditions to reduce the water content to no higher than 8%. One hundred kilograms of the dried fruits are then ground (≥20 mesh) and added to 400 L of sterile water. The mixtures are stirred under sterile conditions at room temperature for twelve hours, and then centrifuged at 1000 rpm to remove insoluble residues. To make the soy bean extract, fresh soy beans are washed and dried under sterile conditions to reduce the water content to no higher than 8%. Thirty kilograms of dried soy beans are then ground into particles of no smaller than 20 mesh, and added to 130 L of sterile water. The mixture is stirred under sterile conditions at room temperature for twelve hours and centrifuged at 1000 rpm to remove insoluble residues. Once the mixed fruit extract solution is

prepared, it is autoclaved at 121°C for 30 minutes and cooled to below 40°C before use.

One thousand grams of the activated yeast powder prepared as described above (Section V, *supra*) is added to 1000 L of the mixed fruit extract solution, and the yeast solution is transferred to the first container (A) shown in Fig. 2. The yeast cells are then cultured in the presence of an alternating electric field having a frequency of 12877 MHz and a field strength of about 380-420 mV/cm (e.g., 406 mV/cm) at 28-32°C under sterile conditions for 32 hours. The yeast cells are further incubated in an alternating electric field having a frequency of 12895 MHz and a field strength of 370-390 mV/cm (e.g., 372 mV/cm). The culturing continues for another 12 hours at 28-32°C.

The yeast culture is then transferred from the first container (A) to the second container (B) (if need be, a new batch of yeast culture can be started in the now available the first container (A)), and subjected to an alternating electric field having a frequency of 12877 MHz and a field strength of 430-450 mV/cm (e.g., 438 mV/cm) for 24 hours at 28-32°C. Subsequently the frequency and field strength of the electric field are changed to 12895 MHz and 380-400 mV/cm (e.g., 385 mV/cm), respectively. The culturing process continues for another 12 hours at 28-32°C.

The yeast culture is then transferred from the second container (B) to the third container (C), and subjected to an alternating electric field having a frequency of 12877 MHz and a field strength of 315-335 mV/cm (e.g., 321 mV/cm) for 24 hours. Subsequently the frequency and field strength of the electric field are changed to 12895 MHz and 250-270 mV/cm (e.g., 250 mV/cm), respectively. The culturing continues for another 12 hours.

The yeast culture from the third container (C) can then be packaged into vacuum sealed bottles for use as medication or dietary supplement, e.g., in the form of health drinks, pills, or powder, etc. If desired, the final yeast culture can also be dried within 24 hours and stored in powder form. The dietary supplement can be taken three to four times daily at 30~60 ml per dose for a three-month period, preferably 10-30 minutes before meals and at bedtime.

In some embodiments, the compositions of the invention can also be administered intravenously or peritoneally in the form of a sterile injectable preparation. Such a sterile preparation can be prepared as follows. A sterilized health drink composition is first treated under ultrasound (≥18,000 Hz) for 10 minutes and then centrifuged for another 10 minutes. The resulting supernatant is adjusted to pH 7.2-7.4 using 1 M NaOH and subsequently filtered through a membrane (0.22 μm for intravenous injection and 0.45 μm for peritoneal injection) under sterile conditions. The resulting sterile preparation is submerged in a 35-38 °C water bath for 30 minutes before use. In other embodiments, the compositions of the invention may also be formulated with pharmaceutically acceptable carriers to be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, suspensions or solutions.

The yeast compositions of the present invention are derived from yeasts used in food and pharmaceutical industries. The yeast compositions are thus devoid of side effects associated with many conventional pharmaceutical compounds.

VII. Examples

In order that this invention be more fully understood, the following examples are set forth. These examples are for the purpose of illustration only and are not to be construed as limiting the scope of the invention in any way.

The activated yeast compositions used in the following examples were prepared as described above, using *Saccharomyces cerevisiae* Hansen AS2.16 cells, cultured in the presence of an alternating electric field having the electric field frequency and field strength exemplified in the parentheses following the recommended ranges listed in Section IV, *supra*. Control (i.e., untreated) yeast compositions were those prepared in the same manner as described in Section VI, *supra*, except that the yeast cells were cultured in the absence of EMFs. All compositions of interest were administered to the animals by intragastric feeding, unless otherwise specified.

Example 1: Effects of Treatment on Renal Failure in Rats

To test the ability of the EMF-treated AS2.16 cells to ameliorate renal failure and restore renal function, forty healthy male Wistar rats (3-6 months

old, about 180 to 200 g body weight) were selected and randomly divided into four equal groups, Groups A, B, C and D. Group D rats were used as controls. Under anesthesia with amobarbital (3.0 ml/100 g body weight), each of Groups A, B and C rats was laid prone on an operating table and its posterior abdominal cavity was opened under sterile conditions. The right kidney was exposed and two thirds of the cortical tissue (about 0.45-5.0 g) of the right kidney were removed. After bleeding was stopped, the muscular tissue was injected with penicillin ($1.5x10^4$ units/100 g body weight) to prevent infection. The wound opening was then closed by stitches. One week later, blood samples were collected from the tail and the carotid artery of each rat in the four groups ten hours after feeding with water only. Blood urea nitrogen (BUN) and serum creatinine levels in the blood samples were determined for all rats. Urine samples from each rat in the four groups were collected for a twenty-four hour period, during which the rat was given water but no food. The collected urine samples were preserved with xylene and the proteinuria concentration in the samples was determined. Subsequently, the abdominal cavity of each of Groups A, B and C rats was re-opened by the same method as described above. The renal pedicel was ligated with a ligature and the left kidney was removed. All rats in the four groups were then raised with normal feed for another week.

At week three, a composition of interest (1.5 ml/100g body weight) was administered to each of the operated rats once daily for the next eight weeks. Rats in Group A were each given the activated yeast composition at a dose of 1.5 ml/100g body weight. Rats in Groups B and C were treated in the same manner as those in Group A, except that they were given the control yeast composition and tap water, respectively, in lieu of the activated yeast composition. Rats in Group D were treated in the same manner as those in Group C, except that the former were not operated on. Urine samples were collected for a twenty-four hour period and the proteinuria concentration was determined. BUN levels and serum creatinine readings in the blood samples were also determined as described above. The results were summarized in Tables 2 and 3.

Table 2. Urine Secretion of Male Wistar Rats.

Grp.	Urine (ml, for a 24 hour period)		Proteinuria (mg, for a 24 hour period)	
	Prior to Administration (week 2)	After Administration (week 11)	Prior to Administration (week 2)	After Administration (week 11)
A	8.3±2.3	25.6±3.5	9.3±3.3	6.7±3.4
B	11.3±3.4	11.7±3.3	11.2±3.8	18.6±6.4
C	11.2±3.2	11.6±3.6	10.9±4.1	19.3±5.7
D	5.1±2.2	9.4±2.9	5.2±2.2	5.8±3.6

Table 3. Serum BUN and Creatinine Levels of Male Wistar Rats.

Group	BUN (mM)		Serum Creatinine (mM)	
	Prior to Administration	After Administration	Prior to Administration	After Administration
A	19.6±4.4	7.6±0.7	103.6±12.4	46.3±7.7
B	18.4±4.3	19.7±3.8	99.6±14.2	169.8±23.4
C	18.8±3.3	17.9±3.6	103.4±14.5	172.3±22.8
D	7.5±0.6	3.8±2.9	26.7±4.4	33.7±10.6

The above results show that the activated yeast composition increased urine secretion, decreased proteinuria concentration, and lowered BUN and serum creatinine levels. In contrast, the control yeast composition demonstrated no such effects.

Additionally, rats given the activated yeast composition showed noticeable improvement in the amount of food intake which resulted in an increase in body weight.

Example 2: Diuretic Effect in Rabbits

To test the diuretic effect of the EMF-treated AS2.16 cells, each of eighteen healthy domesticated male rabbits (*Oryctolagus curiculus*, 3-5 months old, about 2±0.2 kg body weight) was injected with 5% glucose saline (10 ml/kg) through the marginal vein of its ear. A urinary catheter was gently inserted into the rabbit's bladder for 8-10cm. The bladder was emptied and urine was collected twice, each for a period of 5 to 10 minutes. The collected urine samples were

measured and recorded. Another catheter was subsquently inserted into the rabbit's stomach. The rabbits were then randomly divided into three equal groups.

A composition of interest (12 ml/kg) was fed to each rabbit through the catheter to the stomach. Rabbits in Group A were each given the activated yeast composition at a dose of 12 ml/kg body weight. Rabbits in Groups B and C were treated in the same manner as those in Group A, except that they were given the control yeast composition and saline, respectively, in lieu of the activated yeast composition. Urine samples were collected every 30 minutes for three times starting 30 minutes after the administration. These results were summarized in Table 4.

Table 4. Effects of Treatment on Urine Secretion.

Group	Urine (ml)			
	Before Administration (0 minutes)	After Administration		
		30 minutes	60 minutes	90 minutes
A	0.6±0.3	7.8±2.6	12.7±1.9	13.8±2.2
B	0.6±0.3	2.4±1.7	3.7±2.3	3.9±2.1
C	0.7±0.2	2.3±1.4	3.5±2.1	4.1±1.9

The above results show that the activated composition increased urine secretion compared to the controls.

While a number of embodiments of this invention have been set forth, it is apparent that the basic constructions may be altered to provide other embodiments which utilize the compositions and methods of this invention.